Bond. (A. K.)

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PHENYL-HYDRAZIN TEST FOR SUGAR IN URINE, AS APPLIED BY ULTZMANN.

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ALTHOUGH the determination of the presence or absence of grape sugar in urine is, as a rule, easy, yet we find now and then cases in which it is extremely difficult to decide by means of the ordinary tests whether the results which we obtain are caused by grape sugar or not.

The fermentation test and the polariscope are unreliable where the percentage of sugar is very small. The reduction tests, the best of which is that of Fehling, are reliable in all cases as *negative* tests—when no reduction is effected no sugar is present; but, as *positive* tests, they fail when the amount of sugar is very small, for, in this case, the results may be due to other reducing substances in the urine. It is with pleasure, therefore, that we read of the introduction of a test of an entirely different order which promises to be a certain *positive* test for sugar in the urine. I refer to the phenyl-hydrazin chloride test, which was introduced by Emil Fischer,[1] and has been applied to the examination of pathological urine by P. Grocco,[2] and by R. von Jaksch.[3]

[1] Berichte der Deutsch Chem. Gesellsch. zu Berlin, 17, 579.
[2] Annali di Chim. appl. alia Farmacie, 79, 258.
[3] Mittheilungen des Wiener Med. Doctoren-Collegiums, 10 Band. See, also, his book, Klinische Diagnostik Innerer Krankheiten.

In this phenyl-hydrazin chloride test the sugar itself is obtained in a crystalline compound, the formula of which is $C_{18}H_{22}N_4O_4$. This compound, which Fischer names phenyl-glukosazon, appears in the form of a yellow sediment which, under the microscope, is seen to consist of fine yellow needles. According to Fischer phenyl-glukosazon is not affected by watery solutions of alkalies. Suspended in warm water, it reduces alkaline copper solutions energetically. It dissolves in hot alcohol, and crystallizes out, when water is added, in the form of yellow needles. Heated to 204°C., it melts to a dark red liquid, and at a higher temperature it is decomposed. Crystals, resembling those formed by glucose, are obtained from other members of the sugar group—levulose, galactose, sorbin, milk sugar, and maltose—but I find[1] that of these only levulose and milk sugar are ever found in the urine. Levulose is found only when dextrose is present, and milk sugar only during the course of the so-called milk fever. All observers agree that these sugars are the only substances which, under the conditions present, yield such crystals. The studies of Fischer, Grocco, and Jaksch have made this test an extremely valuable one in the hands of skilled investigators and chemists, but the addition of water to the urine interferes with the exactness of the results, and the use of the water-bath is quite unnecessary.

It is my purpose in this paper to describe a simple and rapid mode of using this test, which I learned from Professor Robert Ultzmann at his clinical lectures in Vienna, in the winter of 1885–86. I do not think that Dr. Ultzmann has yet published any-

[1] Neubauer und Vogel, Analyse des Harns, 1881.

thing upon the subject, but the matter is of such importance that I do not hesitate to quote his words.

My notes upon his lectures are as follows: "It is a very simple test, and the most exact of all. Put into a test tube 1 cm. high of the phenyl-hydrazin chloride, and upon it 1 cm. (a little less than this) of crystals of sodium acetate. Then add urine to the half-test tube. Boil the mixture till it becomes clear. Let it stand for fifteen minutes, and examine the sediment for crystals. If albumen is present, you must first coagulate and filter. The urine must be clear. If there is no sugar, we find no such crystals. These crystals are in yellow sheaves, unlike any other product which appears in urine. Tyrosin gives crystals of a somewhat similar shape, but they are not yellow, and appear in urine only on rare occasions, as in acute yellow atrophy of the liver. This tyrosin, too, is a natural, not an artificial, product. Leucin, which has at times a somewhat similar shape, is unlike in color.

"This method is also useful for determining approximately the *quantity* of sugar in the urine."

Here, then, was a method which seemed in simplicity and accuracy to be exactly what is needed for the practising physician's use.

I procured some of the phenyl-hydrazin chloride in Vienna, and since my return from Europe I have made a very careful study of the test as applied by Ultzmann (for convenience, I shall speak of it as "Ultzmann's method"). The result is that I fully endorse his recommendation of it, although, in some respects, my experience has been different from his.

In my experiments I have used no appliances save those which are within the reach of every physician. Yet, in determining the sensitiveness of the test, I

have exercised the greatest care in the preparation and measurement of my standard solutions of sugar.

I make the test as follows: I pour the phenyl salt—which is a dry substance resembling bran—into an empty test-tube until the tube is filled to the distance of about four-tenths of an inch from the bottom, and add crystals of sodium acetate, ground fine, to an equal height. Upon this I pour the urine—clear or cloudy—until the tube is half full. This gives in a test-tube five inches long, about the following proportions in weight: 1 part phenyl salt, 2 parts sodium acetate, 15 parts urine. I shake the tube until the crystals of sodium acetate are dissolved, then heat gently over a low flame until the mixture boils, and boil it for about half a minute—whether it becomes clear or not makes no difference. I then cover the tube and let it stand, and, after a proper interval, examine the sediment with the microscope. If sugar is present there will be seen first fine, bright yellow needles, which branch out or are joined by others as they are formed, until the field is dotted with groups like delicate sprays or sheaves, or radiating from a centre. A magnifying power of 200 diameters is sufficient for this study. That the phenyl salt is always in excess when the test is made in this way is shown by the constant presence of reddish globules in the field.

My experiments fall naturally into two series. The first series includes those made for the purpose of finding how long the mixture must stand before the crystals appear; of determining what percentage of sugar is necessary for the formation of the crystals; and of comparing this test, as simplified, with Fehling's test, which I believe to be the best heretofore in use. For this series I used, instead of pure

grape sugar, which cannot easily be weighed, a crystalline compound of sodium chloride and grape sugar, which is easily weighed. A solution of this salt in distilled water (containing, by calculation, 1 part of anhydrous glucose to 1250 of water) gave, by Ultzmann's method, after standing eighteen minutes, a sediment in which, by the aid of the microscope, the crystals could be seen grouping themselves into delicate, spray-like forms. The same mixture yielded, twenty-one hours later, a yellow sediment, which was seen, with a one-inch objective as a hand-lens, to consist of feather-like groups of yellow, needle-shaped crystals. Under the microscope abundant sheaves of yellow crystals were seen. A mixture containing 1 part of sugar to 2000 parts of normal urine, which gave a fair reaction for sugar by Fehling's test, yielded, by Ultzmann's method, on standing twenty-two hours, four or five large yellow sheaves to a field of the microscope, and after it had stood forty-eight hours, about a dozen sheaves to a field. Mixtures containing 1 part sugar to 3000 or 4000 parts normal urine, which gave, with Fehling's fluid, very uncertain tests, yielded, by Ultzmann's method, after twenty-two hours, no crystals; but twenty-three hours later, several well-formed sheaves to a slide. It is possible that still more dilute solutions of sugar in urine might yield the crystals when treated in this way, but for ordinary office-use greater accuracy than this is not needed.

The second series includes those experiments made to determine whether, under any circumstances, the test, as applied by Ultzmann, fails to give the crystals in urine which contains more than one-fortieth per cent.—1 part to 4000 parts—of grape sugar. In these experiments I used solutions

of known strength of very pure grape sugar in distilled water. I treated by Ultzmann's method in all fourteen mixtures of these standard solutions with fresh, non-saccharine urine containing from one-thirty-sixth to one-half per cent. grape sugar, and obtained the yellow crystals in every case. In eleven of them the crystals appeared within twenty-four hours after the test was made; in three of them when the mixtures had stood more than twenty-four hours. Several of these fourteen mixtures were made with urine which threw down phosphates on boiling; one with urine which yielded, on standing before tested, a very abundant sediment of urates; one with urine containing a considerable quantity of albumen—the yellow crystals in this case in part appearing in the masses of coagulum, in part settling from the supernatant fluid in the form of sheaves; one with urine to which I had added quite an excess of sodium bicarbonate, to find whether alkalinity of the urine interferes with the test—in this case brisk effervescence occurred from the action of the bicarbonate upon the phenyl salt, but, as the latter was in excess, the solution continued acid and the crystals appeared, though in smaller numbers than usual.

These tests with artificial solutions of glucose in urine were confirmed by similar tests with the urine of patients suffering from diabetes mellitus.

Many tests were made with normal urine, but in none of them could I find even a single yellow crystal or sheaf.

Through the kindness of a medical friend, I had the opportunity of testing, by Ultzmann's method, a very interesting specimen of urine[1] which contains

[1] Described by Dr. Bruen, Boston Med. and Surg. Journal, December 30, 1886, and January 27, 1887; and by Dr. Marshall, THE MEDICAL NEWS, January 8, 1887.

a substance which was mistaken for sugar by several life insurance examiners. This urine behaved with the phenyl-hydrazin test exactly like normal urine.

In the sediment from a solution of sugar in hydrant water, tested by Ultzmann's method, a yellow plant was found under the microscope, which in its very earliest stages resembled somewhat in color and form small sprays of sugar crystals. Upon further observation, however, the peculiar manner of growth of the plant removed all doubt upon the subject. This plant was not found in any of the urine mixtures.

I will now state briefly the results of my work :

The test of Fischer, as applied by Ultzmann, is easy to make, and does not take more time than Fehling's test, if, as is necessary, the latter be made with fresh solutions.

It is suitable for office use by physicians, and may be accurately made by those whose training in chemistry is imperfect.

It does not require great skill in the use of the microscope. In all my examinations the crystals, if present, were found upon the first slide examined.

When the sugar present amounts to one-fifteenth per cent., the crystals may be found fifteen minutes after the test is made.

The test is about as sensitive in the presence of sugar as that of Fehling, and is not liable to be disturbed by other substances contained in the urine. It is obtained in alkaline as well as in acid, in cloudy as well as in clear urine.

Normal urine does not respond to the test.

If the crystals are found, the proof of the presence of sugar is absolute.

My experiments lead me to believe that in *all* cases in which sugar is present in the urine to the amount of one-fortieth per cent. or more, the crys-

tals may be found—although it may be necessary, when the quantity of sugar is very small, to let the mixture stand after boiling as many as forty-eight hours.

The single crystals show a strong tendency, in the most dilute as well as the most concentrated solutions of sugar, to form clusters; so that, in solutions which have stood some time, only the characteristic clusters may be found.

The crystals or clusters undergo no change of importance in aqueous solutions for a month or more, and so any sediment may be preserved and inspected from time to time. Even in urine mixtures they remain for weeks unchanged, decomposition of the mixtures being prevented by the phenyl salt.

Ultzmann's method may be used for rough *quantitative* determinations. In case the urine contains one-tenth per cent. or more of sugar, we may estimate the amount from the quantity of yellow crystalline sediment, even when the urine is not clear. When less than one-tenth per cent. is present, the amount may be roughly estimated from the number of crystals in a field of the microscope.

The salt of phenyl-hydrazin used for the test is quite stable, retaining its properties for months (apparently for years), even when exposed frequently to the air.[1]

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[1] Phenyl-hydrazin chlor. hydrat. may be obtained from Eimer & Amend, 205 Third Avenue, New York.

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